

In re U.S. Patent Application of:
Antrim et al.

Filed: June 23, 2003

For: Dextrinized, Saccharide-
Derivatized Oligosaccharides

Group Art Unit 1623

Examiner: Devesh Khare

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Perminus Mungara, declare as follows:

1. My name is Perminus Mungara. I am submitting this Declaration in support of the captioned patent application.
2. I received my Ph.D in Chemistry from the University of Connecticut in 1995. Before that, I received a Masters Degree (1993) and a Bachelors Degree (1986) from the University of Nairobi, Kenya.
3. I am presently employed as a Research Scientist at Grain Processing Corporation of Muscatine, Iowa. I have held this position since 2005. Prior to that, I was employed for five years as an Assistant Scientist at the Department of Food Science and Human Nutrition at Iowa State University. From August 1996 to May 2000 I was a post-doctoral research associate at the Iowa State University. I have held a number of other teaching, research and lecturing positions.
4. I am a member of numerous professional societies, including the American Chemical Society, the American Oil Chemist Society, and the American Association of Cereal Chemists.

5. I have authored or co-authored 21 peer-reviewed journal articles. I am a named inventor in two U.S. Patents (U.S. Patent 6,632,925 and 6,806,353).
6. I have been asked to review the subject matter of the present application and to compare the subject matter to the art cited in the Office Action of February 7, 2007.
7. Specifically, I have been asked to review the Meyers et al. and Fouache references, and to comment on whether exemplary products prepared in accordance with the present invention differ from the products disclosed in the Meyers et al. and Fouache references.
8. I prepared four products to exemplify products prepared in accordance with the present invention. Specifically, I extruded a saccharide product having an average degree of polymerization ranging from 1 to 4 with a mixture of malto-oligosaccharides, the derivatization being catalyzed with an acid. These products will be identified herein as Products 1 through 4.
9. Product 1 was prepared by extruding MALTRIN M200 and citric acid, at a ratio of 99:1 MALTRIN: citric acid. MALTRIN M200 is a syrup solid sold by Grain Processing Corporation of Muscatine, Iowa. The temperature in the extruder barrel was 200°C.
10. Product 2 was prepared by extruding MALTRIN M200, dextrose, and citric acid, at a ratio of 94:5:1 MALTRIN: dextrose: citric acid. The barrel temperature was 190°C.
11. Product 3 was prepared by extruding a mixture of MALTRIN M180, dextrose, and citric acid, at a ratio of 89:10:1 MALTRIN: dextrose: acid. MALTRIN M180 is a syrup solid sold by Grain Processing Corporation of Muscatine, Iowa. The barrel temperature in the extruder was 190 °C.
12. Product 4 was prepared by extruding MALTRIN M250 and acid, at a ratio of 99:1 M250: acid. MALTRIN M250 is a syrup solid sold by Grain Processing Corporation of Muscatine, Iowa. The barrel temperature in the extruder was 200°C.
13. The Meyers et al. reference discusses a product known as Fibersol. I caused to be obtained a commercial sample of Fibersol.
14. The Fouache et al. reference discloses a product known commercially as NUTRIOSE. I caused to be obtained a commercial sample of NUTRIOSE.

15. Products 1-4 and the commercial NUTRIOSE and Fibersol products were evaluated.
16. Specifically, these products were evaluated for taste, 4 hour digestibility, viscosity, dextrose equivalence, pH, residual dextrose, and ash.
17. The "taste" data was evaluated qualitatively via sampling of a 10% aqueous mixture of each product by a panel that included myself. The remaining tests are quantitative tests.
18. Specifically, the 4 hour digestibility test was performed in accordance with an internal Grain Processing Corporation procedure. This procedure was established based in part on articles by Muir and O'Dea, in *Am. J. Clin. Nutr.* (56:123-127 (1992) and 57: 40-56 (1993), respectively). This procedure is an *in vitro* digestion assay for starch and related carbohydrates.
19. Viscosity was as measured at 30% solids and 30°C using GPC internal method S-46, a method that specifies a Rapid Visco Analyzer (RVA). Dextrose equivalence was determined in accordance with GPC internal test method C-21. Residual dextrose and pH were determined by conventional methods. Ash was determined via thermogravimetric analysis using a TGA 2950 (TA Instruments, Inc., Newcastle, Delaware).
20. The results of these tests are provided in Table 1 hereinbelow.

TABLE 1

Sample	Taste (10% solution)	4-hour digestibility	Viscosity @30% solid/30°C (cps)	DE	pH	Residual Dextrose (%)	Ash (%) (By TGA)
1	Slight tartness but agreeable taste	33	24	17.8	4.6	4.9	1.2
2	Slight sweetness	42	21	19.4	4.9	5.6	0.9
3	Slight sweetness	38	24	19.6	4.8	6.1	1.0
4	Bland taste	38	25	11.5	4.8	2.5	0.8
Fibersol-2	Bland taste	4	15	13.5	3.2	0.4	0.1

NUTRIOSE	Bland taste	15	25	3.5	3.0	0.2	0.5
----------	-------------	----	----	-----	-----	-----	-----

CA = citric acid, Dex = dextrose

21. As seen, the digestibility of the four products prepared in accordance with the present invention was substantially greater than that of the Fibersol and NUTRIOSE products. Other properties also demonstrated a marked difference between the four products of the present invention and the Fibersol and NUTRIOSE products.
22. I was also asked to perform a methylation analysis of the NUTRIOSE and Fibersol products and of two products of the invention, specifically products 1 and 3. Such an analysis was done under my direction. The methylation analysis was performed in accordance with the procedures outlined in Hakomori, S. I., *J. Biochem (Tokyo)* (1964) 55:205-08, as modified by Kim et al., *Carbohydrate Research* (2006) 341:1061-64.
23. Generally, in the methylation analysis, the material subject to analysis is exhaustively methylated using methyl iodide. This converts the free hydroxyl group to methyl ether. The methylated product is then exhaustively hydrolyzed with acid catalysis. This creates a hydroxyl group at places where a glycosidic bond once existed. The product was then analyzed and the positions of the hydroxyl groups then determined. This represents where linkage points existed in the original products.
24. For instance, "t-glc" indicates a terminal glucose group. Other indications are similar; for instance, "3,4-glc" indicates where a branch point had existed at the 3- and 4- positions.
25. Of interest is the 4-glc and 6-glc data. 4-glc data indicates where a 1-4 glycosidic bond had existed, and the 6-glc data indicates where a 1-6 glycosidic bond had existed. The 4-glc is of particular relevance, because the alpha 1-4 glycosidic bonds are the most digestible, followed by alpha 1- 6. The methylation data did not differentiate between alpha and beta bonds.
26. The results are shown in the following Table 2.

TABLE 2

	NUTRIOSE	Fibersol	Sample 1	Sample 3
linked-glc	% Total Area*	% Total Area*	% Total Area*	% Total Area*
t-glc	19.7	21.1	23.9	21.9
2-glc	3.0	3.7	2.7	2.1
3-glc	4.4	4.2	3.7	3.1
4-glc	26.0	28.8	35.0	42.6
6-glc	12.5	13.7	10.1	7.7
3,4-glc	4.4	3.3	2.8	2.9
2,3-glc + 2,4-glc	3.1	3.5	3.2	3.2
4,6-glc	16.0	14.6	12.7	12.2
2,6-glc + 3,6-glc	4.8	3.4	2.3	1.6
2,3,4-glc	1.1	0.5	0.6	0.5
3,4,6-glc	3.2	1.8	1.9	1.1
2,4,6-glc	1.9	1.4	1.1	1.0
2,3,6-glc**	nd	nd	nd	nd
Total Area %	100.1	100.0	100.0	99.9

27. As seen, the two products evaluated had more 1,4 and 1,6 bonds in the original product than did the Fibersol and NUTRIOSE products. This data strongly suggests that the products of the invention were more digestible than the commercial products. This is confirmed by the digestibility data reported in Table 1 hereinabove. I note that the total 1,6 bond content is within the range specified in the Fouache et al. patent.

28. I also caused to be measured the weight average molecular weights of these products. They were as follows:

TABLE 3

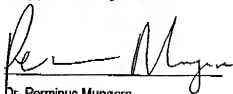
	NUTRIOSE	Fibersol	Sample 1	Sample 3
Mw	4841	3205	3306	3093

29. This data demonstrates that the Fibersol and NUTRIOSE products are different from the four products evaluated herein, and from the disclosures of the Fouache et al. and Meyers et al. references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date:

August 7, 2007

A handwritten signature in dark ink, appearing to read 'Perminus Mungara', written over a horizontal line.

Dr. Perminus Mungara